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Deficient production of lysyl oxidase in cultures of malignantly transformed human cells

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Lysyl oxidase activity in the culture medium of eight malignantly transformed human cell lines was very low compared with that in four control fibroblast lines, being 9–16% in five sarcoma cell lines and 7–11% in three other tumour cell lines. The low enzyme activity was probably due to deficient enzyme synthesis rather than impaired secretion into the cell medium, as low activity was also found in urea extracts of the cell pellets. Lysyl oxidase production thus appears to be closely regulated with deficient collagen gene expression in malignant transformation.

Lysyl oxidase *Transformation* *Collagen* *Elastin*

1. INTRODUCTION

Cells transformed by tumour viruses or chemical carcinogens and spontaneous tumour cells all produce greatly reduced amounts of fibronectin and collagen in culture, due to decreased copy numbers of the respective mRNAs (review [1]). This in turn is primarily caused by a reduced rate of transcription of the corresponding genes [2,3].

Collagen synthesis is characterized by the presence of an unusually large number of post-translational modifications, many of which are unique to collagens and a few other proteins (review [4]). These modifications require at least 8 specific enzymes, 5 of them being intracellular and 3 extracellular, as well as several non-specific enzymes. Activities of the 5 intracellular enzymes have been studied previously in several transformed cell lines [5–7]. Prolyl 4-hydroxylase activity was low in the cell lines studied except in freshly transformed cells, whereas the 4 other enzyme activities showed a highly variable pattern, in that reduced, normal or even markedly high values were found depending on the enzyme and the transformed cell type in question [5–7]. The extracellular conversion of procollagen to collagen has also been found to be impaired in at least some types of transformed fibroblasts, possibly due to a lack of procollagen proteinases [8–10], whereas no data are available on the production of lysyl oxidase by any transformed cells.

Lysyl oxidase (EC 1.4.3.13) is an extracellular copper enzyme that initiates the cross-linking of collagens and elastin by catalyzing oxidative deamination of the ϵ-amino group in certain lysine and hydroxylysine residues [11]. A deficiency in this enzyme activity has recently been found in two human heritable connective tissue disorders characterized by abnormalities in copper metabolism [12–14]. Changes in lysyl oxidase activity in the medium of cultured fibroblasts [12,15] and aortic smooth muscle cells [16] suggest that the enzyme may be regulated, as its activity varies, reaching its maximum at pre-confluency and being responsive to certain changes in the external environment of the cells. Few data are available on such regulation, however. We now report very low levels of lysyl oxidase activity in a number of

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human sarcoma and other tumour cell lines, suggesting that this enzyme activity may be closely regulated with collagen gene expression in malignant transformation.

2. MATERIALS AND METHODS

The cultured human cell lines were locally established embryonal skin (HES) and lung (HEL) fibroblasts, commercially available foreskin fibroblasts (F-7000, Flow Laboratories, Irvine, Scotland), embryonal lung fibroblasts (WI-38, CCL 75 from the American Type Culture Collection, Rockville, MD), SV40 virus-transformed WI-38 cells (VA-13/WI-38, CCL 75.1), fibrosarcoma cells (HT-1080, CCL 121), embryonal rhabdomyosarcoma cells (RD, CCL 136), trophoblastic choriocarcinoma cells (BeWo, CCL 98), another line of choriocarcinoma cells (JEG-3, HTB 36) and melanoma cells (G-361, CRL 1424), and also further adult rhabdomyosarcoma cells (A-204) and fibrosarcoma cells (8387) provided by Drs J.E. DeLarco and G.J. Todaro (Frederick Cancer Research Center, Frederick, MD). The cells were grown in Eagle's minimal essential medium supplemented with 10% foetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 50 μg/ml ascorbate [5]. For the measurement of lysyl oxidase activity, subconfluent cells were washed twice with a solution of 0.01 M sodium phosphate and 0.14 M NaCl, pH 7.4, and then cultured for an additional 24 h period in the above medium without serum but supplemented with 5 mg/ml bovine serum albumin [14,17]. This medium was collected and used for the assays of lysyl oxidase activity. In some experiments the cell pellets were prepared by trypsinization and the enzyme activity was also measured in 4 M urea extracts of the homogenized cell pellets [14]. Special care was taken to avoid comparing density-inhibited control cells with over-growing transformed cells.

Lysyl oxidase activity was assayed in a final volume of 0.6 ml with 0.8×10^6 dpm [6-^{3}H]lysine-labelled purified chick-embryo calvaria collagen as a substrate [14,18]. The substrate was preincubated at 37°C for 60 min to promote fibril formation [18], and the incubation time with the enzyme was then 10 h [14]. The cell culture media, which had been stored at −20°C for up to 3 weeks, were dialyzed for 3 h at 4°C against a solution of 0.14 M NaCl in 0.1 M sodium phosphate, pH 7.8, and aliquots of the dialyzed media were then used for the assays without any prior concentration [14]. The urea extracts of the homogenized cell pellets were dialyzed for 8 h against the same solution.

3. RESULTS AND DISCUSSION

The 5 human sarcoma cell lines studied here (table 1) have previously been shown to produce collagen in reduced amounts as compared with several non-transformed human fibroblast lines (e.g. [5–7]). The type of collagen synthesized varies, the SV40 virus-transformed WI-38 cells and the 8387 cells producing mainly type I collagen, the RD cells type III collagen, the HT-1080 cells type IV collagen, and the A-204 cells type V collagen [19,20]. All the control cell lines synthesized mainly type I collagen.

Lysyl oxidase activity was markedly low in the medium of all 5 sarcoma cell lines (table 1). Significant differences were found even within the control group in that the enzyme activity of the HES cells was significantly higher than of the WI-38 line ($P < 0.05$, t-test) or the F-7000 cells ($P < 0.001$), the difference between the latter two also being significant ($P < 0.01$). Nevertheless, such differences do not influence the main conclusion of markedly low lysyl oxidase activity in the sarcoma cells, as these values were in the range 9–16% of the control mean (table 1). The SV40 virus-transformed WI-38 cells had a lysyl oxidase activity about 12% of that recorded in the corresponding non-transformed cell line WI-38.

The 3 other malignant tumour cell lines, BeWo, JEG-3 and G-361, likewise had very low lysyl oxidase activity, about 7–11% of the control mean (table 1). As the activity values in all the sarcoma and other tumour cell lines are very low, being close to the borderline of detection, it cannot be determined whether any differences exist between the 2 groups of malignantly transformed cells in this respect.

Prolyl 4-hydroxylase activity in the 5 sarcoma cell lines studied here is about 25–65% of that in a number of control fibroblast lines [5–7], the activity in the SV40 virus-transformed WI-38 cells being about 40% of that in the non-transformed

Table 1

Lysyl oxidase activity in the medium of cultured cells

Cell line	n^a	Lysyl oxidase activity	
		dpm/10^6 cells[b]	% of control mean
Control cells			
Embryonal skin fibroblasts (HES)	6	4150 ± 790	
Embryonal lung fibroblasts (HEL)	6	2950 ± 1180	
Embryonal lung fibroblasts (WI-38)	6	3080 ± 580	
Foreskin fibroblasts (F-7000)	7	2260 ± 320	
Sarcoma cells			
SV40 transformed WI-38 cells (Va 13/Wi-38)	3	380 ± 10	12.2
Fibrosarcoma cells (8387)	8	280 ± 100	9.0
Fibrosarcoma cells (HT-1080)	6	490 ± 180	15.8
Embryonal rhabdomyosarcoma cells (RD)	8	390 ± 200	12.5
Adult rhabdomyosarcoma cells (A-204)	8	280 ± 120	9.0
Other transformed cells			
Choriocarcinoma cells (BeWo)	5	210 ± 140	6.8
Choriocarcinoma cells (JEG-3)	8	310 ± 120	10.0
Melanoma cells (G-361)	7	330 ± 230	10.6

[a] Number of independent measurements on separate cell cultures
[b] Mean ± SD

WI-38 cells [5]. The activities of the 4 other specific intracellular enzymes of collagen synthesis vary from about 50 to 220% of the control values, part of this variation probably reflecting differences in the types of collagen synthesized [5–7]. The present data indicate that lysyl oxidase activity is reduced in malignantly transformed cells to a much greater degree than that of any of the specific intracellular enzyme activities.

About 90% of the lysyl oxidase activity in fibroblast cultures is usually found in the medium, an additional 10% being extractable from the cell pellets with 4 M urea [14]. To study whether the low enzyme activity in the malignantly transformed cells is due to deficient secretion of the enzyme, lysyl oxidase activity was also measured in such urea extracts. No accumulation of the enzyme was found in the cell pellets, however, the value in the transformed cells being even lower than in the controls and falling below the limit of accurate assay. It therefore seems that the deficient lysyl oxidase production in malignantly transformed cells is due to a low rate of enzyme synthesis, possibly caused by decreased transcription of the corresponding gene(s), a mechanism that has been demonstrated for transformed cells in the case of low collagen production [2,3]. The present data indicate an efficient regulation of lysyl oxidase activity with the rate of collagen synthesis in malignant transformation. Further work is needed to demonstrate whether a more general phenomenon of coordinated regulation between the lysyl oxidase and collagen production exists.

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REFERENCES

[1] Alitalo, K. and Vaheri, A. (1982) Adv. Cancer Res. 37, 111–158.
[2] Avvedimento, E., Yamada, Y., Lovelace, E., Vogeli, G., De Crombrugghe, B. and Pastan, I. (1981) Nucleic Acids Res. 9, 1123–1131.

[3] Sandmeyer, S., Gallis, B. and Bornstein, P. (1981) J. Biol. Chem. 256, 5022–5028.
[4] Kivirikko, K.I. and Myllylä, R. (1985) Ann. NY Acad. Sci., in press.
[5] Myllylä, R., Alitalo, K., Vaheri, A. and Kivirikko, K.I. (1981) Biochem. J. 196, 683–692.
[6] Pihlajaniemi, T., Myllylä, R., Alitalo, K., Vaheri, A. and Kivirikko, K.I. (1981) Biochemistry 20, 7409–7415.
[7] Majamaa, K., Myllylä, R., Alitalo, K. and Vaheri, A. (1982) Biochem. J. 206, 499–503.
[8] Arbogast, B.W., Yoshimura, M., Kefalides, N.A., Holtzer, H. and Kaji, A. (1977) J. Biol. Chem. 252, 8863–8868.
[9] Sundarraj, N. and Church, R.L. (1978) FEBS Lett. 85, 47–51.
[10] Vaheri, A., Kurkinen, M., Lehto, V.-P., Linder, E. and Timpl, R. (1978) Proc. Natl. Acad. Sci. USA 75, 4944–4948.
[11] Siegel, R.C. (1979) Int. Rev. Connect. Tissue Res. 8, 73–118.
[12] Royce, P.M., Camakaris, J. and Danks, D.M. (1980) Biochem. J. 192, 579–586.
[13] Kuivaniemi, H., Peltonen, L., Palotie, A., Kaitila, I. and Kivirikko, K.I. (1982) J. Clin. Invest. 69, 730–733.
[14] Peltonen, L., Kuivaniemi, H., Palotie, A., Horn, N., Kaitila, I. and Kivirikko, K.I. (1983) Biochemistry 22, 6156–6163.
[15] Layman, D.L., Narayanan, A.S. and Martin, G. (1972) Arch. Biochem. Biophys. 149, 97–101.
[16] Gonnerman, W.A., Ferrera, R. and Franzblau, C. (1981) Biochemistry 20, 3864–3867.
[17] Kuivaniemi, H., Peltonen, L. and Kivirikko, K.I. (1985) Am. J. Hum. Genet. 37, 798–808.
[18] Siegel, R.C. (1974) Proc. Natl. Acad. Sci. USA 71, 4826–4830.
[19] Alitalo, K., Keski-Oja, J. and Vaheri, A. (1981) Int. J. Cancer 27, 755–761.
[20] Alitalo, K., Myllylä, R., Sage, H., Prizl, P., Vaheri, A. and Bornstein, P. (1982) J. Biol. Chem. 257, 9016–9024.